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Oxidative Deamination of Several Amino Acids in Aqueous Solution by Gamma Irradiation*

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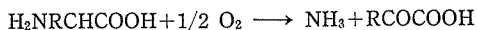
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A general overall reaction of oxidative deamination of amino acid in aqueous solution, $\text{H}_2\text{NRCHCOOH} \longrightarrow \text{NH}_3 + \text{RCOCOOH}$, was characterized on leucine, α -aminobutyric acid and oxaloacetic acid after exposure to γ -irradiation. Keto acids corresponding to the parent amino acids were produced by γ -irradiation and determined quantitatively by chromatography and spectroscopic measurements after being derived to 2,4-dinitrophenylhydrazones.

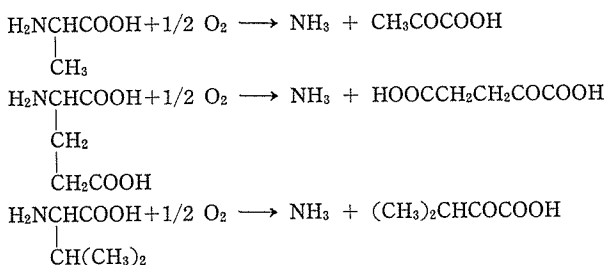
Some spectroscopic and chromatographic properties of the keto acid hydrazones were reported here continuing to the previous reports.

INTRODUCTION

Radiolytic reactions of amino acids in solution were studied by many investigators¹⁻⁶⁾ and, recently, oxidative radiolysis of amino acid, peptides and proteins was studied by Hatano⁷⁻¹⁰⁾ and Garrison *et al.*¹¹⁾. One of the major reactions induced in amino acids in solution by radiation is oxidative deamination to arise ammonia and keto acid corresponding to the parent amino acid under an aerobic condition in terms of a general overall reaction:



In the previous paper⁸⁾ the author described that when the each aqueous solution of alanine, glutamic acid and valine was irradiated with γ -rays the corresponding keto acid, pyruvic acid, α -ketoglutaric acid and α -ketoisovaleric acid respectively, were produced from the parent amino acid accompanying by liberation of ammonia as follows:



The further study¹⁰⁾ showed the several ultraviolet spectra of 2,4-dinitro-

* The material in this paper was presented at the 4th Symposium on Isotope in Japan, May 1962, The Proceedings, R-35, p. 401.

** 波多野博行, 六鹿 宗治

phenylhydrazones of many keto acids proposed to be produced from irradiated amino acids in solution such as glyoxalic acid produced from glycine, oxaloacetic acid from aspartic acid, isopropylpyruvic acid from leucine, phenylpyruvic acid from phenylalanine, β -hydroxyphenylpyruvic acid from tyrosine, β -hydroxypyruvic acid from serine, α -keto- β -hydroxybutyric acid from threonine, β -mercaptopyruvic acid from cysteine, α -keto- γ -methiolbutyric acid from methionine, α -keto- δ -guanidinovaleric acid from arginine, α -keto- ϵ -aminocaproic acid from lysine, β -imidazolylpyruvic acid from histidine, β -indolpyruvic acid from tryptophan and α -keto- δ -aminovaleric acid from proline.

In this paper more further experiments were presented on separation and characterization of oxaloacetic acid, isopropylpyruvic acid, and α -ketobutyric acid, which were produced from γ -irradiated aspartic acid, leucine, and α -aminobutyric acid respectively, in aqueous solution under the aerobic condition.

EXPERIMENTAL

Materials. Leucine and aspartic acid used in this experiments were a product of Ajinomoto Co. Ltd., Tokyo, α -aminobutyric acid was that of Tokyo Kasei Co. Ltd., Tokyo. All were chromatographic pure grade. Authentic specimen of isopropylpyruvic acid, methylethylpyruvic acid and oxaloacetic acid was prepared and kindly supplied by Dr. H. Katsuki of Kyoto University, Kyoto⁽¹²⁾. Reagent 2,4-dinitrophenylhydrazine was at a concentration of 1 mg per ml in 2 *N* hydrochloric acid.

Methods. Irradiation of γ -rays: A two-kilocurie cobalt-60 γ -ray irradiation facility⁽¹³⁾ was used for γ -irradiation. The dose rate was approximately 1.4×10^5 r. per hour.

Spectrophotometric measurements of keto acid 2,4-dinitrophenylhydrazones: The keto acid 2,4-dinitrophenylhydrazones was prepared by the method described in the previous papers^(8,11). Spectrophotometric measurements were carried out on the preparation of the keto acid derivatives using a Beckman DU spectrophotometer and a Hitachi EPS-2 selfrecording spectrophotometer according to the procedure of the previous papers^(8,11).

Chromatography of the keto acid 2,4-dinitrophenylhydrazones: Paperchromatography of the keto acid 2,4-dinitrophenylhydrazones were performed according to the method of Katsuki⁽¹²⁾ as follows: Toyo filter paper No. 51 was immersed into a mixture of ether and diethylphthalate (80 : 20 v/v) and dried in air as soon as possible. The treated filter paper was used for reverse phase chromatography on which a 0.1 *N* sodium bicarbonate solution was used as a developing solvent. For ordinary paperchromatography a usual method described in the previous report⁽⁸⁾ was used. An untreated Toyo filter paper No. 51 and a developing solvent of *n*-butanol, ethanol and 0.1 *N* sodium carbonate (4 : 1 : 2, v/v) or filter paper treated with a 0.1 *N* Na_2CO_3 solution and a developing solvent of *n*-butanol, ethanol, 0.1 *N* Na_2CO_3 (10 : 3 : 10, v/v) were used in these experiments.

Quantitative determination of the α -keto acids was carried out successfully using Hyflo-Super-Cel column chromatography and spectrophotometric measure-

ments by the method described in the previous papers⁸⁾.

RESULTS

(1) **Ultraviolet and visible absorption spectra of 2, 4-dinitrophenylhydrazones of the α -keto acids.** After exposure to γ -irradiation of a 320 kiloroentgen dose the aqueous solutions of α -aminobutyric acid, isoleucine, leucine and aspartic acid were reacted with the 2,4-dinitrophenylhydrazine reagent to produce the hydrazones of carbonyl compounds. In the resulted mixture it was found that produced α -ketobutyric acid, isopropylpyruvic acid, and oxaloacetic acid which was followed by a secondary product, pyruvic acid, were derived respectively to the hydrazones.

Fig. 1 showed the ultraviolet and visible absorption spectra of 2,4-dinitrophenylhydrazones of α -ketobutyric acid and isopropylpyruvic acid, which were

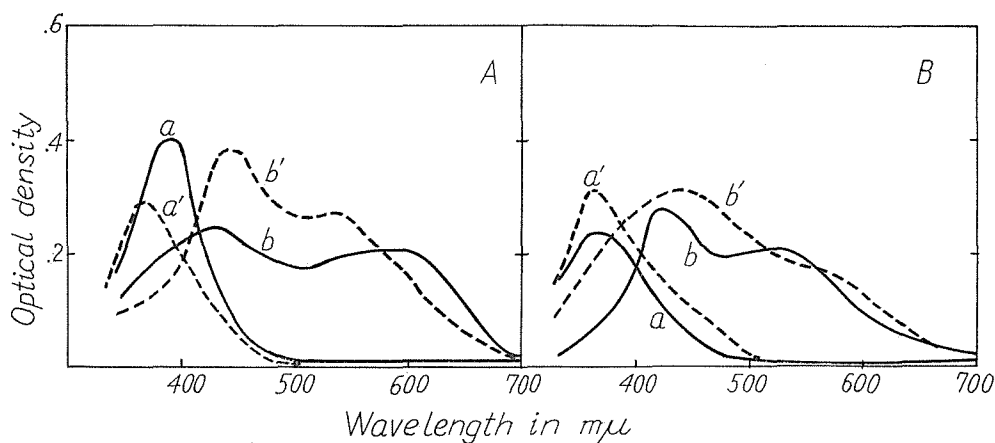


Fig. 1. Absorption spectra of 2,4-dinitrophenylhydrazones of the α -keto acids produced from the γ -irradiated solutions of the parent amino acids, measured in alkaline solutions.

A: The hydrazone of α -ketobutyric acid produced from γ -rayed α -aminobutyric acid.

B: That of isopropylpyruvic acid produced from γ -rayed leucine, a: cis-isomer in 0.5 N Na_2CO_3 ; a': trans-isomer in 1 N Na_2CO_3 ; b: cis-isomer in 0.5 N Na_2CO_3 and 0.5 N NaOH ; b': trans-isomer in 0.5 N Na_2CO_3 and 0.5 N NaOH .

The 10 mM solutions of the amino acid were irradiated with γ -rays of 320 kr. dose.

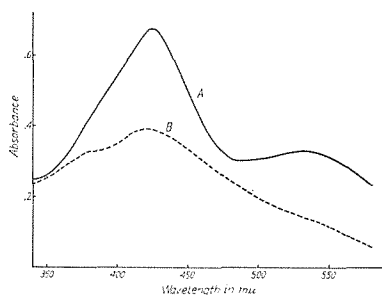


Fig. 2. Absorption spectra of 2,4-dinitrophenylhydrazones of γ -irradiated isoleucine and of an authentic specimen (A) of methylethylpyruvic acid (B) in an alkaline solution.

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produced from the parent amino acids respectively. Methylethylpyruvic acid, however, failed to be showed in the irradiated isoleucine solution as shown as Fig. 2.

(2) **Paperchromatography of 2,4-dinitrophenylhydrazones of the keto acids.** Good separation and identification of cis- and trans- isomers of isopropylpyruvic acid and α -ketobutyric acid 2,4-dinitrophenylhydrazones were carried out using the ordinary and the reverse phase chromatographies. Oxaloacetic acid derivative arise no isomers of the derivatives. Rf values of the paperchromatography were tabulated in Table 1.

Table 1. Paperchromatographic characterization of keto acid 2,4-dinitrophenylhydrazones which were derived from γ -irradiation products of amino acids in aqueous solutions.

2, 4-dinitrophenylhydrazone	Rf	
	Ordinary method	Reverse phase method
Pyruvic acid authentic trans-isomer	.45	.56
Pyruvic acid authentic cis-isomer	.53	.35
Oxaloacetic acid authentic	.37	.71
Irradiated aspartic acid	.38, .44, .52	.75, .55
Isopropylpyruvic acid authentic trans-isomer	.60	—
Isopropylpyruvic acid authentic cis-isomer	.70	—
Irradiated leucine	.57, .74**	—
α -Ketobutyric acid authentic trans-isomer*	.48	.64
α -Ketobutyric acid authentic cis-isomer*	.56	.33
Propion aldehyde authentic	.28	—
Irradiated α -aminobutyric acid	.55, .44, .29	.62, .37

All Rf values were mean obtained from 15-20 experiments.

* A solvent, n-butanol : ethanol=10 : 3 : 10 and filter papers treated with a 0.01N NaHCO₃ solution were used. Spot of propion aldehyde derivative coloured brown, different from other ordinary colour of yellow.

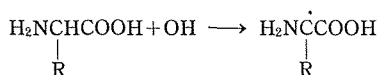
** More two spot appeared at Rf .33 and .22 which were unknown.

*** More one spot was found at Rf .52, which was also uncharacterized.

(3) **Column chromatography of 2,4-dinitrophenylhydrazones of the keto acid.** Quantitative separation and isolation of cis- and trans-isomers of the keto acid derivatives were performed by the column chromatography using a Hyflo-Super-Cel column. Fig. 3 shows the chromatograms of the keto acid derivatives. A secondary product from oxaloacetic acid was identified with pyruvic acid of which 2,4-dinitrophenylhydrazone have the cis- and trans- and trans-isomers.

DISCUSSION

In the previous paper it was proposed that the radiolytic deamination of amino acid to produce the corresponding keto acid should be proceed through a radical mechanism of indirect action of radiation as follows :



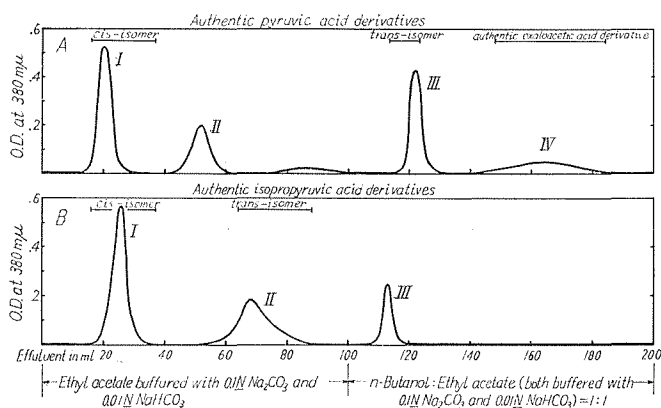


Fig. 3. Column chromatographic separation of keto acid 2,4-dinitrophenylhydrazones which were derived from γ -irradiation products of amino acids in aqueous solutions. A: A chromatogram of 2,4-dinitrophenylhydrazones of radiation products produced from an irradiated aspartic acid solution.

Peak I: cis-isomer of pyruvic acid 2,4-dinitrophenylhydrazone

Peak II: uncharacterized

Peak III: trans-isomer of pyruvic acid 2,4-dinitrophenylhydrazone

Peak IV: oxaloacetic acid 2,4-dinitrophenylhydrazone

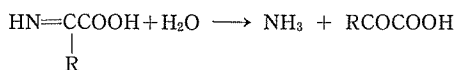
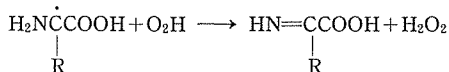
B: That of an irradiated leucine solution.

Peak I: cis-isomer of isopropylpyruvic acid 2,4-dinitrophenylhydrazone

Peak II: trans-isomer of isopropylpyruvic acid 2,4-dinitrophenylhydrazone

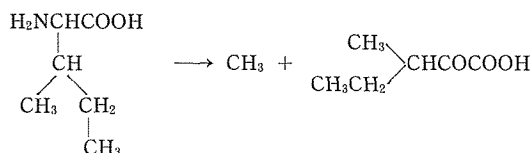
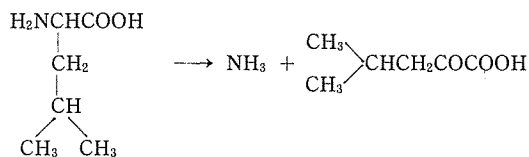
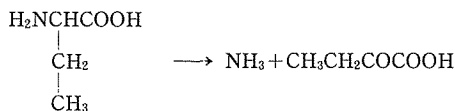
Peak III: uncharacterized

Hyflo-Super-Cel column, 9 mm \times 210 mm was used.



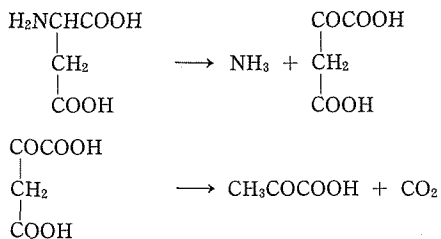
Three keto acids had been characterized and results obtained in this experiment added further four examples to this radiolytic oxidation of amino acid.

Each amino acid would be perhaps deaminated by the indirect action of γ -radiation through the mechanism over all as follows:

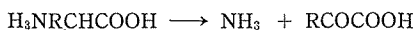


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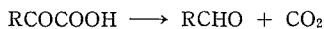
Oxaloacetic acid, however, was further rendered to decomposition owing to its extraordinary instability arising pyruvic acid and carbon dioxide spontaneously as follows :



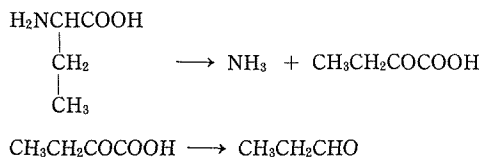
The pyruvic acid was confirmed by the chromatographic evidences. Methylethylpyruvic acid proposed above to be produced from irradiated isoleucine could not be detected spectroscopically and chromatographically in this experiment. Though the identification of many other keto acids was not always successful easily owing to their instability and radiosensitivity, it appears to be certainly in general that an amino acid may be deaminated oxidatively by the radiation products of water in γ -irradiated aqueous solutions to produce the corresponding keto acid and ammonia according to the following overall reaction :



Furthermore, these keto acids may be changed to the corresponding aldehydes by spontaneous decarboxylation or a radiation-induced reaction, as follows :



Propionaldehyde produced from α -ketobutyric acid was detected on the paper-chromatogram of irradiation products of α -aminobutyric acid, as presented in Table 1, according to the following reaction :



In the peptides and proteins described previously, the resulting mixtures of these keto acids and aldehydes have been investigated using the methods of spectroscopic measurements and chromatographic separation of 2,4-dinitrophenylhydrazones of these carbonyl compounds^{7,10}. Volatile aldehydes and other fragmental compounds may be detected by a gaschromatographic procedure.

ACKNOWLEDGMENTS

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